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Effect of Nonionizing Radiation on the Purkinje Cells of the Uvula in Squirrel Monkey Cerebellum

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Effect of Nonionizing Radiation on the Purkinje Cells of the Uvula in Squirrel Monkey Cerebellum

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Pregnant squirrel monkeys were exposed to 2450-MHz (CW) microwaves at an equivalent power density of 10 mW/cm^2 (SAR 3.4 mW/g) for three hours daily in a cavity-cage module. The exposure began when pregnancy was determined by a hormonal method, and continued through the offspring's first 9.5 months. After irradiation, the brains of the offspring were fixed with formaldehyde, and the inferior vermis of each cerebella was removed and processed for histologic observations. Purkinje cell density in the uvula was determined in sagittal serial sections. There was no significant difference between control and experimental animals in the number of Purkinje cells per mm of Purkinje cell line (linear density), as well as in the density of Purkinje cells in the Purkinje cell layer.

Key words: monkey cerebellum, Purkinje cells, nonionizing radiation, microwaves

INTRODUCTION

Rugh et al [1974, 1975], Shore et al [1977], Berman et al [1978], and others have reported fetal resorption, exencephaly, and fetal death when pregnant rats and mice were exposed to 2450-MHz (CW) microwave radiation. Only Berman et al [1978] exposed animals at low power densities (3–10 mW/cm^2). The above-mentioned studies were designed to examine whether the prescribed exposure conditions would cause gross teratologic effects or other macroscopic changes. These studies did not investigate subclinical and/or subtle changes in the central nervous system.

A recent study designed to explore the subtle effects of 2450-MHz and 100-MHz (CW) nonionizing radiation on the developing rat cerebellum revealed a significant decrease in the relative number of Purkinje cells in the developing cerebella of rats irradiated in utero, or during early postnatal life. The observed de-

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crease in Purkinje cells was permanent if fetuses were irradiated in utero, but the effect was reversible when irradiation was confined to early postnatal life [Albert et al, 1981].

This study reports the effect of 2450-MHz (CW) microwave radiation on the density of Purkinje cells in the cerebellar uvula of the squirrel monkey *(Saimiri Sciureus)*. The uvula was chosen for analysis because the mean value of the cell density in various parts of the cerebellar cortex is essentially equivalent to the mean value of the uvula alone [Lange, 1972, 1975]. Therefore, an enormous amount of technical and quantitative work can be saved by performing Purkinje cell counts on the uvula only instead of on the entire cerebellum.

MATERIALS AND METHODS

Fourteen pregnant squirrel monkeys *(Saimiri Sciureus)*, and then the mothers and their offspring, were sham-exposed or exposed to 2450-MHz (CW) microwaves at a power density equivalent to 0 or 10 mW/cm^2 (SAR 0 or 3.4 mW/g) for three hours/day, five days/week. The animal's were irradiated in a cavity-cage module at SRI International* (for details see Heynick et al [1977]).

Onset of pregnancy in the squirrel monkeys was confirmed by a hormonal assay. Whole-body irradiation in pairs began on the thirty-fifth day of gestation and continued throughout gestation. After birth, the mother and infant as dyads were irradiated for an additional nine months. The SAR was determined on saline-filled dolls by calorimetric calibration techniques.

After the end of the irradiation period, microwave (five male and two female) and sham (four male and three female) irradiated offspring were transported by air to The George Washington University where they were euthenized within 24 hours. The animals were weighed and anesthetized with ketamine hydrochloride (60 mg/kg). Their brains were fixed by cardiac perfusion with 10% buffered formalin. Two to three hours after fixation, the brains were removed, weighed, and their volumes were determined. The cerebella were dissected, and the inferior vermis was accurately separated from the rest of each cerebellum by two sharp cuttings through the paravermal furrows. The inferior vermis of each cerebellum was further processed for paraffin embedding. The tissues were serially sectioned at 10-μ thickness in the sagittal plane and were stained with hematoxylin-eosin.

Equal numbers of uvual from sham-exposed and irradiated animals were treated in the same manner, and in pairs, to eliminate errors due to artifacts of dehydration, sectioning, and cutting.

The density of Purkinje cells was estimated by determining first the area of the "sheet" of the Purkinje cell layer, and second, the total number of Purkinje cells in this area, according to Braitenberg and Atwood, 1958; Armstrong and Schild, 1970; Palkovits et al, 1971; Mlonyeni, 1973 . The Purkinje cell density was expressed as number of the cells/mm^2 of the Purkinje cell layer. The density was

*The authors are indebted to Dr. Joel Kaplan of SRI International for the exposures and cooperation in meeting the difficult time requirements of our experimental schedule.

also expressed as the number of Purkinje cells/mm of the Purkinje cell line (linear density).

The spatial extent of the array of Purkinje cells in the uvula was determined by projecting onto a sheet of paper every 30th section, including the first and the last complete sections. The Purkinje cell line of the uvula only was drawn (Fig. 1). The lengths of the resulting lines were measured with a calibrated map measuring wheel (curvimeter). Thus, values $l_1, l_2, l_3, \ldots, l_n$ were obtained. Usually, seven to eight sections constituted the entire width of the uvula between complete sections (2.10–2.40 mm). The total area of the Purkinje cell "sheet" was derived by summing $(l_1 + l_2)/2$, $(l_2 + l_3)/2$, $(l_{n-1} + l_n)/2$ and multiplying by the distance between the sections ($l_1, l_2, \ldots, l_n$ = the total number of sections counted).

The number of Purkinje cells of the uvual in each section was counted under light microscopy. In parasagittal sections, Purkinje cells lie virtually in one single layer. The total number of Purkinje cells of each uvula between the complete parasagittal sections was counted by summing $(N_1 + N_2)/2$, $(N_2 + N_3)/2$, $\ldots$, $(N_{n-1} + N_n)/2$ and multiplying by the distance between the sections ($N_1, N_2, \ldots, N_n$ = the number of Purkinje cells found in each section, n = the number of sections in which cells were counted). Finally, the mean cell density was calculated for each uvual of the control and the experimental animals.

Only those cells that were present at the interface between the molecular and the granular layers, and that displayed a visible nucleolus, were counted. Purkinje

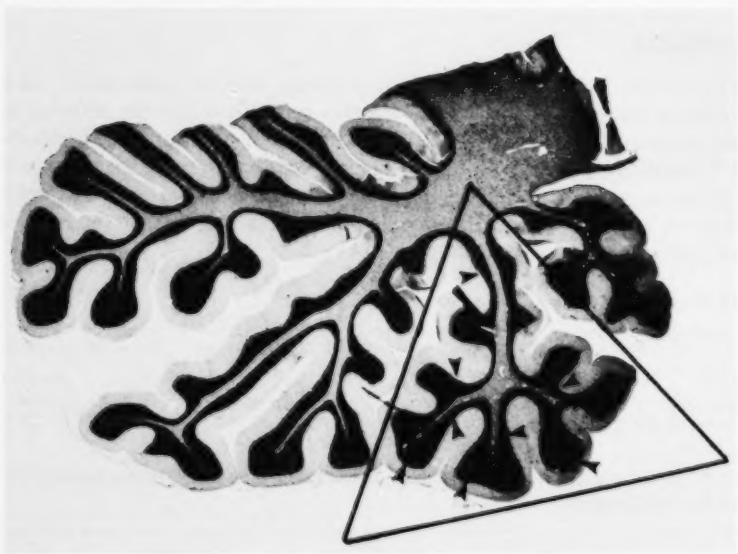

Fig. 1. A macrophotograph of a sagittal section of the inferior vermis of the squirrel monkey cerebellum. Enclosed in the triangle is the uvula. The arrows point to the interface (Purkinje cell line) between the molecular and granular layers.

cells within the molecular and granular layers were not counted. No correction formulas for nucleolar fragmentation [Agduhr, 1941; Floderus, 1944; Abercrombie, 1946; Marrable, 1962] were used in this study, since a) the same calculations were applied for the data obtained from both control and experimental animals, and b) all correction formulas inevitably lead to an underestimation because they assume that even the smallest fragments of the nucleoli are visible [Ebbeson and Tang, 1965; Cammermeyer, 1967; Mlonyeni, 1973].

RESULTS

The average body mass of the animals, and the average mass and volume of their brains, were approximately the same among control and experimental animals (Table 1).

The effects of irradiation on the absolute and relative number of Purkinje cells are summarized in Table 2. All values referred to in the tables are the mean values of all parasagittal sections of the control and experimental animals. Since these data showed a normal symmetrical distribution, they were analyzed by Student's t-test. Table 2 shows that there was no statistically significant difference between control and experimental animals in:

1) Mean total surface of Purkinje cell "sheet".
2) Mean number of Purkinje cells/mm of Purkinje cell line.
3) Mean total number of Purkinje cells in the uvula.
4) Mean density of Purkinje cells/mm^2 of Purkinje cell sheet.

DISCUSSION

The results of these experiments demonstrate that the mean density of Purkinje cells in the uvula of the squirrel monkey cerebellum was not affected by chronic exposure to 2450-MHz (CW) fields at 10 mW/cm^2 for three hours daily (SAR 3.4 mW/g). It should be clearly pointed out that in this experiment, the irradiation commenced on the 35th day of pregnancy, and continued throughout gestation. After birth, the offspring were further irradiated at the same exposure parameters for 9.5 months.

TABLE 1. Mass and Volume of Monkeys and Their Brains

	Whole-body mass (g)	Brain mass (g)	Brain volume (cm^3)
Control	481.85 ± 22.95	29.46 ± 0.69	28.20 ± 1.78
Experimental	507.43 ± 10.57	28.53 ± 0.64	27.90 ± 1.34

TABLE 2. Effect of 2.45-GHz (CW) Irradiation on the Purkinje (P-) Cell Density in the Uvula of Squirrel Monkey Cerebellum

	P-cell "sheet" (mm^2) mean ± SEM	P-cells/mm of P-line mean ± SEM	Total number of P-cells mean ± SEM	P-cells/mm^2 of P-cell mean ± SEM
Control	53.41 ± 2.79	7.35 ± 0.18	39,711 ± 2,021	744.68 ± 17.43
Experimental	49.36 ± 2.80	7.39 ± 0.15	36,712 ± 1,930	746.16 ± 13.68

In another study [Albert et al, 1981], we observed that exposure of pregnant rats to 2450-MHz (CW) fields at 10 mW/cm² for 21 hours daily for five days (average SAR 2.0 mW/g) resulted in a significant decrease in cerebellar Purkinje cells. In the same study, another group of rats, provided by the Environmental Protection Agency, displayed a similar decrease in Purkinje cells. These rats were exposed to 100-MHz (CW) fields at 46 mW/cm² for four hours per day, five days per week (SAR 2.77 mW/g) throughout pregnancy, and then their offspring exposed for another 90 days. These animals were euthenized 14 months after the last exposure. Although the average SAR values were slightly lower in exposed rats than in exposed monkeys, Purkinje cell decrease occurred only in the former species. These observations suggest that the observed effects on Purkinje cells might be independent of frequency and average SAR values. Other factors that might contribute to the discrepant results between the two species might be different geometrical configurations of the animals, particularly those of the head, method of exposure (free-field versus multipath), length of gestation, length of exposure per day, anatomical differences in the structures covering the cerebellum, depth of penetration, species differences, and others. Which of these factors are important is not clear. One might assume that 2450 MHz is closer to resonant frequency for the rat than for the squirrel monkey. It can also be argued that the rat gestation period is short in comparison with that of the monkey. Therefore, the magnitude of effect would be much greater in a contracted period when many developmental processes are occurring rapidly in the rat. The same developmental processes take place over a more lengthy time in the monkey.

Other important anatomical features affecting depth of penetration of radiation may also be responsible for the observed differences. In the rat, the cerebellum is exposed in the dorsal and posterior aspect, and is covered by a thin calvaria. In the squirrel monkey, the cerebellum is overlapped by the occipital lobes, thicker calvaria and thick muscles in the cervical regions (Fig. 2). Such considera-

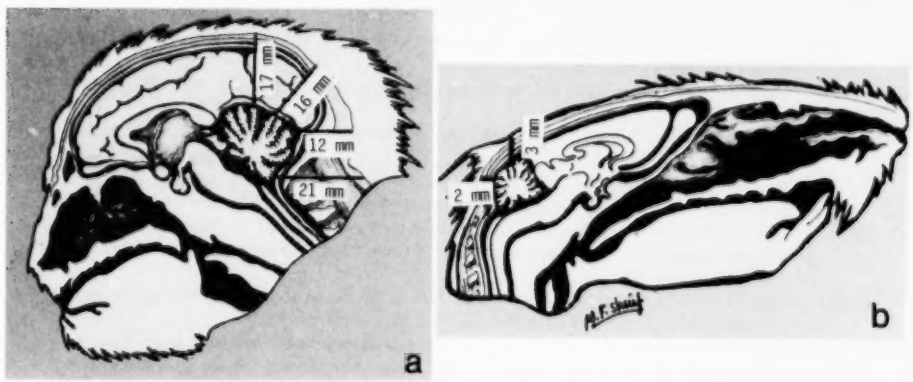

Fig. 2. a) Diagram of a sagittal section of the head and upper cervical region of squirrel monkey. The depth of various parts of the cerebellum is indicated. b) Similar diagram in the rat showing the exposed dorsal and posterior aspects of the cerebellum.

tions may necessitate measurements of local or regional rather than whole body SAR values for proper evaluation of results. The possibility of specific results cannot be ruled out. The effect of 2450-MHZ (CW) radiation on other parts of the monkey brain is under investigation.

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